

WE CLAIM:

1. A method for detecting the relative amounts of at least two mRNA sequences in at least one biological sample, the method comprising:
 - 5 (a) isolating mRNA from at least one biological sample;
 - (b) amplifying at least two mRNA transcripts from each biological sample to produce amplicons, wherein the amplicons are less than about 300 bases in length, and wherein the amplification comprises a linear amplification step;
 - 10 (c) electronically hybridizing the amplicons produced in step (b) to at least two probes bound to a support at predetermined locations; and
 - (d) detecting the amounts of each amplicon hybridized to the bound probes at the predetermined locations.
2. The method of claim 1, wherein the relative amounts of at least two mRNA sequences are detected in at least two samples, wherein each set of amplicons produced for each sample in step (b) is selectively electronically hybridized to an electronically
 - 15 controlled set of predetermined locations on the support.
3. The method of claim 2 wherein all sets of amplicons for each sample are electronically hybridized prior to the detection step (d).
4. The method of claim 2 wherein the relative amounts of at least two mRNA sequences
 - 20 are detected in at least 10 samples.
5. The method of claim 4 wherein the relative amounts of at least two mRNA sequences are detected in at least 50 samples.
6. The method of claim 1 wherein the relative amounts of at least 5 mRNA sequences are detected.
- 25 7. The method of claim 1 wherein the relative amounts of at least 10 mRNA sequences are detected.
8. The method of claim 1 wherein the relative amounts of at least 20 mRNA sequences are detected.
9. The method of claim 1 wherein the relative amounts of at least 40 mRNA sequences
 - 30 are detected.

10. The method of claim 1 wherein the relative amounts of at least 50 mRNA sequences are detected.
11. The method of claim 1 wherein total mRNA is isolated from the biological sample in step (a).
- 5 12. The method of claim 1 wherein polyA mRNA is isolated from the biological sample in step (a).
13. The method of claim 1 wherein the amplification step (b) comprises a reverse-transcription step in which a cDNA library is generated from the mRNA isolated in step (a).
- 10 14. The method of claim 13 wherein the amplification in step (b) further comprises a DNA polymerase amplification step in which members of the cDNA library are amplified with at least two chimeric primers, wherein each chimeric primer comprises a RNA polymerase recognition site upstream of a sequence specific for one of the mRNA sequences of interest.
- 15 15. The method of claim 14 wherein the DNA polymerase amplification step is linear.
16. The method of claim 15 wherein the amplification in step (b) further comprises a geometric DNA polymerase amplification step, in which members of the cDNA library are amplified with at least one additional primer, wherein at least one primer is complementary to a portion of each mRNA sequence of interest and at least one
20 primer is the same as a portion of each mRNA sequence of interest.
17. The method of claim 16 wherein the additional primer is a set of random primer sequences.
18. The method of claim 16 wherein the additional primer is a polydeoxythymine primer
25 sequence.
19. The method of claim 16 wherein the additional primer is a set of primer sequences specific for each mRNA sequence of interest.
20. The method of claim 14 wherein the DNA polymerase amplification step is geometric, in which members of the cDNA library are amplified with at least one additional

primer, wherein at least one primer is complementary to a portion of each mRNA sequence of interest and at least one primer is the same as a portion of each mRNA sequence of interest.

- 5 21. The method of claim 20 wherein the additional primer is a set of random primer sequences.
22. The method of claim 20 wherein the additional primer is a polydeoxythymine primer sequence.
- 10 23. The method of claim 20 wherein the additional primer is a set of primer sequences specific for each mRNA sequence of interest.
24. The method of claim 14 wherein the chimeric primers are labeled with an affinity moiety.
25. The method of claim 24 wherein the affinity moiety is biotin.
- 15 26. The method of claim 25 wherein the amplification in step (b) further comprises immobilizing the DNA produced in the DNA polymerase amplification step on a surface comprising a protein selected from the group consisting of streptavidin and avidin.
27. The method of claim 14 wherein the amplification in step (b) further comprises *in vitro* transcription of the DNA produced in the DNA polymerase amplification step.
- 20 28. The method of claim 14 wherein the amplified mRNA sequences of interest comprise a type IIs endonuclease recognition site.
29. The method of claim 28 wherein the amplification in step (b) further comprises a type IIs endonuclease digestion step.
- 25 30. The method of claim 29 wherein the amplification in step (b) further comprises an *in vitro* transcription step.
31. The method of claim 1 wherein the amplification in step (b) comprises an *in vitro* transcription step.

32. The method of claim 1 wherein each amplicon is between about 50 and about 300 nucleotides in length.
33. The method of claim 1 wherein each amplicon is between about 50 and about 200 nucleotides in length.
- 5 34. The method of claim 1 wherein each amplicon is between about 50 and about 100 nucleotides in length.
35. The method of claim 1 wherein the amplicons differ in length by less than 20 bases.
36. The method of claim 1 wherein the amplicons differ in length by less than 10 bases.
- 10 37. The method of claim 1 wherein the detection in step (d) is by an enzymatic extension of the probes to which the amplicons are hybridized to incorporate a labeled nucleotide.
38. The method of claim 1 wherein the detection in step (d) is by detecting a labeled nucleotide incorporated into the amplicons.
39. The method of claim 1 wherein the detection in step (d) is by
- 15 i) electronically or passively hybridizing a labeled reporter probe to the amplicon hybridized to the bound probe, and
- ii) detecting the labeled reporter probe.
40. The method of claim 1 wherein the probes are bound to a permeation layer over an electrode of a semiconductor chip device.
- 20 41. The method of claim 1 wherein the detection in step (d) is by fluorometry, colorimetry, or luminometry.
42. The method of claim 41 wherein the detection is by fluorometry.
43. A method of preserving and reusing a nucleic acid library produced from a patient biological sample, the method comprising:
- 25 (a) isolating mRNA from a patient biological sample;
- (b) reverse-transcribing the mRNA from step (a) to produce a cDNA library;
- (c) amplifying the cDNA library from step (b) by a DNA polymerase reaction utilizing at least one chimeric primer comprising a RNA polymerase recognition site

upstream of a sequence specific for a mRNA transcript of interest and a fill-in primer for the complementary nucleic acid strand chosen from the group consisting of sequence specific primers and random primers, wherein at least one of the primers used is chosen from the group consisting of 5' affinity-moiety labeled chimeric primers and 5' affinity-moiety labeled sequence specific fill-in primers;

(d) binding the amplification products from step (c) to a solid support coated with an affinity-binding moiety;

(e) utilizing the bound amplification products from step (d) as a template for an *in vitro* transcription reaction;

(f) separating the *in vitro* transcription products from step (e) from the amplification products bound to the solid support; and

(g) utilizing the bound amplification products from step (f) as a template for at least one additional *in vitro* transcription reaction, wherein the amount of *in vitro* transcription product produced is not significantly less than that produced in step (e).

44. The method of claim 43 further comprising repeating steps (f) and (g).

45. The method of claim 44 further comprising repeating steps (f) and (g).

46. The method of claim 45 further comprising repeating steps (f) and (g).

47. The method of claim 43 wherein the amplified sequence comprises a type IIs endonuclease recognition site, the method further comprising the step of digesting the amplification products with a type IIs endonuclease prior to the *in vitro* translation of step (e).

48. The method of claim 43 wherein the fill-in primer is a sequence specific primer.

49. The method of claim 43 wherein the fill-in primer is a random sequence primer.

50. The method of claim 43 wherein the affinity moiety is selected from the group consisting of biotin, haptens, and an antigenic moiety.

51. The method of claim 43 wherein the affinity moiety is biotin.

52. The method of claim 51 wherein the affinity-binding moiety is selected from the group consisting of streptavidin and avidin

53. The method of claim 43 wherein the solid support is selected from the group consisting of paramagnetic beads, polymer beads, and metallic beads.

54. A method of detecting the extent of hybridization of a nucleic acid in a sample to a probe nucleic acid sequence, the method comprising:

5 (a) electronically hybridizing the nucleic acid in a sample to a nucleic acid probe bound to a support at a predetermined location;

(b) utilizing the hybridized nucleic acid as a template in a nucleic acid polymerase reaction to extend the bound probe, whereby a labeled nucleotide is incorporated into the extended probe; and

10 (c) detecting the labeled nucleotide incorporated into the extended bound probe at the predetermined location.

55. The method of claim 54 wherein the labeled nucleotide comprises a labeling moiety selected from the group consisting of fluorescent moieties, colorigenic moieties, chemiluminescent moieties, and affinity moieties.

15 56. The method of claim 55 wherein the labeled nucleotide comprises a fluorescent moiety.

57. The method of claim 54 wherein the nucleic acid polymerase reaction is a DNA polymerase reaction.

20 58. The method of claim 54 wherein the nucleic acid polymerase reaction is a reverse-transcriptase reaction.

25 59. A method of providing an internal control for an individual test site in a nucleic acid hybridization reaction assay to determine the presence of at least one nucleic acid sequence of interest in at least one nucleic acid containing sample, wherein the nucleic acid hybridization assay is performed on an electronically controlled microarray comprising at least two test sites, the method comprising:

(a) attaching a mixed nucleic acid probe consisting of a first nucleic acid probe specific for a first nucleic acid sequence known to be present in the sample, and a second nucleic acid probe specific for a second nucleic acid sequence of interest to a first test site on the electronically controlled microarray;

(b) attaching a mixed nucleic acid probe consisting of the first nucleic acid probe and a third nucleic acid probe specific for a third nucleic acid sequence of interest, wherein the third nucleic acid sequence of interest may be the same as or different than the second nucleic acid sequence of interest, to a second test site on the electronically controlled microarray;

(c) electronically hybridizing the sample nucleic acids from at least one sample to the nucleic acid probes on the first and second test sites;

(d) specifically detecting the extent of hybridization of the sample nucleic acids to the first nucleic acid probe at the first and second test sites;

(e) specifically detecting the extent of hybridization of the sample nucleic acids to the second and third nucleic acid probes at the first and second test sites;

(f) comparing the hybridization values obtained for the first nucleic acid probe at the first and second test sites to obtain a normalization factor; and

(g) normalizing the hybridization values obtained in (e) for the second and third probes using the normalization factor obtained in (f).

60. The method of claim 59 wherein the first nucleic acid sequence is a sequence which encodes, or is complementary to a sequence which encodes, a housekeeping gene.

61. The method of claim 59 wherein the first nucleic acid sequence is an exogenous nucleic acid sequence which has been added to the sample.

62. The method of claim 59 wherein the specific detection in (d) is by detecting a labeled nucleotide which has been specifically incorporated into the sample nucleic acids which contain the first nucleic acid sequence by a nucleic acid polymerase reaction.

63. The method of claim 59, further comprising the step of electronically or passively hybridizing a first reporter nucleic acid comprising a detectable moiety specific for the first nucleic acid sequence to the sample nucleic acids which are hybridized to the first nucleic acid probe at the first and second test sites, wherein the specific detection in (d) is by detecting the detectable moiety.

64. The method of claim 59, further comprising the step of extending the first nucleic acid probe by utilizing the sample nucleic acids which have hybridized to the first nucleic acid probe as a template for a nucleic acid polymerase reaction, wherein the specific detection in (d) is by detecting a labeled nucleotide which has been incorporated into the extended first nucleic acid probe by the polymerase reaction.

65. The method of claim 64, wherein the first nucleic acid probe attached in (a) comprises a protecting group that prevents enzymatic extension of the probe.

66. The method of claim 59 wherein the specific detection in (e) is by detecting a labeled nucleotide which has been specifically incorporated into the sample nucleic acids which contain the second and third nucleic acid sequences of interest by a nucleic acid polymerase reaction.

67. The method of claim 59, further comprising the step of electronically or passively hybridizing a second reporter nucleic acid probe comprising a detectable moiety to the sample nucleic acids which are hybridized to the second nucleic acid probe at the second test site, wherein the second reporter probe is specific for the second and third nucleic acid sequences of interest, and wherein the second reporter probe contains one or more reporter probe sequences, , wherein the specific detection in (e) is by detecting the detectable moiety.

68. The method of claim 59, further comprising the step of extending the second and third nucleic acid probes by utilizing the sample nucleic acids which have hybridized to the second nucleic acid probe as a template for a nucleic acid polymerase reaction, wherein the specific detection in (e) is by detecting a labeled nucleotide which has been incorporated into the extended second and third nucleic acid probes by the polymerase reaction.

69. The method of claim 68, wherein the second nucleic acid probe attached in (b) comprises a protecting group that prevents enzymatic extension of the probe.

70. The method of claim 59, wherein a first detectable moiety is detected in step (d), and a second detectable moiety is detected in step (e).

71. The method of claim 70 wherein the first and second detectable moieties are independently selected from the group consisting of fluorescent moieties, colorigenic moieties, chemiluminescent moieties, and affinity moieties.
72. The method of claim 71 wherein the first and second detectable moieties are fluorescent moieties.

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